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# MICROBIAL DEGRADATION AND ACCUMULATION OF PESTICIDES IN AQUATIC SYSTEMS

Doris F. Paris, et al

Environmental Protection Agency
Athens, Georgia

January 1975

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The microbial degradation and sorption of carbaryl, malathion, butoxyethyl ester of 2,4-dichlorophenoxyacetic acid (2,4-DBE), methoxychlor, atrazine, diazinon, captan, parathion, and toxaphene were investigated. Malathion and 2,4-DBE were found to undergo transformation readily in both bacterial and fungal cultures. Degradation of malathion and 2,4-DBE at low concentrations (< 1 mg/l) in batch cultures of bacteria followed second-order kinetics as predicted by the Michaelis-Menten theory. A single isomer, β-monoacid of malathion, was the primary metabolite in transformation of malathion by both bacterial and fungal populations. The major metabolite found in 2,4-DBE studies was 2,4-D. Carbaryl underwent chemical hydrolysis to α-naphthol in both heterogeneous bacterial cultures and uninoculated controls. In the cultures α-naphthol was metabolized to 1,4-naphthoquinone and two unidentified compounds. Bacterial degradation of methoxychlor was slower than bacterial degradation of malathion or 2,4-DBE. The insecticide was metabolized to methoxychlor-DDE. Rapid and extensive sorption of pesticides to fungi, bacteria, and algae was observed with methoxychlor and toxaphene, but not with any of the other pesticides investigated. Distribution coefficients for methoxychlor ranged from $1.2 \times 10^3$ to $4.8 \times 10^4$ for the different organisms whereas the coefficients for toxaphene ranged from $3.4 \times 10^3$ to $1.7 \times 10^4$. Captan underwent neither microbial degradation nor sorption because of its rapid hydrolysis in water.

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# MICROBIAL DEGRADATION AND ACCUMULATION OF PESTICIDES IN AQUATIC SYSTEMS

by

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This report has been reviewed by the National Environmental Research Center--Corvallis, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

## PREFACE

The support and assistance of Dr. Walter M. Sanders III, Chief, Freshwater Ecosystems Branch, Southeast Environmental Research Laboratory, U. S. Environmental Protection Agency, Athens, Georgia, are acknowledged with sincere thanks.

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## SECTION I

## INTRODUCTION

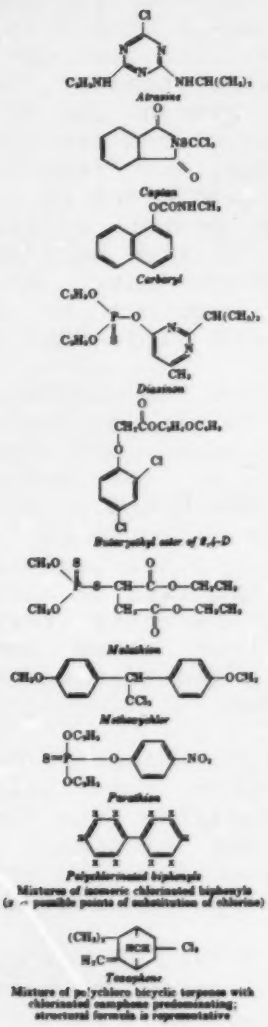
The use of pesticides increases each year as the world population and demand for food increases. In 1970 alone, 1,034 billion pounds of active ingredients of pesticides and related products were produced in the United States (1). For 1972 the National Soils Monitoring Program (2) reports a significant increase in the use of the most common pesticides, in particular atrazine, captan, malathion, and 2,4-D, over figures quoted for 1970 (3). This increased usage of pesticides has raised the amount of these compounds reaching streams, rivers, and reservoirs by unintentional (runoff, groundwater) additions or intentional (dumping and spraying of waters for pests) additions. It has become important, therefore, to know more about the effects of the pesticides on the environment. An evaluation of their environmental impact requires an understanding of their breakdown processes, both biological and non-biological.

Pesticides may affect the environment in several ways. A pesticide with a slow rate of degradation will persist in the environment, stimulating some populations and supressing others. An imbalance in the ecosystem results. Other pesticides will degrade rapidly, some to products that are more toxic than the parent compound and some to harmless products. Microorganisms are commonly believed to be a key factor in determining the fate of many pesticides in aquatic systems; however, a literature review (4) revealed few studies concerning the rates and products of either microbial or chemical degradation of some of the most commonly used pesticides.

Ten pesticides were selected (Table 1) for degradation studies in 1971. Since then polychlorinated biphenyls (PCB's) have been excluded from the studies. Our studies were concerned with the microbial degradation of these pesticides; a complementary project (5) focused on the chemical and photochemical degradation.

Caution must be observed in applying laboratory derived microbial degradation rates to natural systems. Although the bacterial populations used in the studies approximated total populations present in natural waters, our cultures were screened to include only those bacteria that degrade the pesticide; only a small fraction of the natural population would be expected to degrade the pesticide. Also, degradation rates may differ because nutrient

## Table 1. PESTICIDES SELECTED FOR STUDY



*Atrazine*

*Captan*

*Carbaryl*

*Diazinon*

*Butoxyethyl ester of 2,4-D*

*Malathion*

*Methoxychlor*

*Parathion*

*Polychlorinated biphenyls*
Mixtures of isomeric chlorinated biphenyls
(x = possible points of substitution of chlorine)

*Toxaphene*
Mixture of polychloro bicyclic terpenes with chlorinated camphene predominating; structural formula is representative

conditions of the laboratory system are not exactly the same as those of a natural system. Similarly, rates of fungal degradation observed in the laboratory would be realistic for only that portion of the fungal population active in the degradation of pesticides. However, the laboratory microbial rates provide a basis for comparison with photochemical and chemical degradation rates, yielding an insight into the competition of the different processes in the environment. Laboratory studies also provide information about the products to be expected.

Sorption of pesticide by aquatic microorganisms affects the distribution of the compounds within an aquatic system. Organisms sorb pesticides, die, and become a part of the sediment. Pesticides in bottom sediments may be recycled to overlying waters through fall and spring inversions or through release of pesticides from the sediments. The sorbed pesticides may also be degraded anaerobically (6) or they may move up the food chain.

At present sufficient information on microbial sorption of pesticides in aquatic systems is not available to predict to what extent these compounds will be sorbed. Environmental factors and the characteristics of organisms and pollutants must be studied to determine their effects on sorption.

The purpose of this research was to study the action of classes of microorganisms (bacteria, fungi, and algae) on the selected pesticides. The investigations included two areas:

- rates and products of degradation of pesticides by microorganisms; and
- sorption of pesticides by microorganisms.

## SECTION II

## SUMMARY

The microbial degradation and sorption of carbaryl, malathion, butoxyethyl ester of 2,4-dichlorophenoxyacetic acid (2,4-DBE), methoxychlor, atrazine, diazinon, captan, parathion, and toxaphene were investigated.

Malathion and 2,4-DBE were found to undergo transformation readily in both bacterial and fungal cultures. Degradation of malathion and 2,4-DBE at low concentrations (< 1 mg/l) in batch cultures of bacteria followed second-order kinetics as predicted by the Michaelis-Menten theory. A single isomer, β-monoacid of malathion, was the primary metabolite in transformation of malathion by both bacterial and fungal populations. The major metabolite found in 2,4-DBE studies was 2,4-D.

Carbaryl underwent chemical hydrolysis to α-naphthol in both heterogeneous bacterial cultures and uninoculated controls. In the cultures α-naphthol was metabolized to 1,4-naphthoquinone and two unidentified compounds.

Bacterial degradation of methoxychlor was slower than bacterial degradation of malathion or 2,4-DBE. The insecticide was metabolized to methoxychlor-DDE.

Rapid and extensive sorption of pesticides to fungi, bacteria, and algae was observed with methoxychlor and toxaphene, but not with any of the other pesticides investigated. Distribution coefficients for methoxychlor ranged from $1.2 \times 10^3$ to $4.8 \times 10^4$ for the different organisms whereas the coefficients for toxaphene ranged from $3.4 \times 10^3$ to $1.7 \times 10^4$.

Captan underwent neither microbial degradation nor sorption because of its rapid hydrolysis in water.

## SECTION III

## CONCLUSIONS

1. Under conditions found in most aquatic environments (pH 5.6-8.0) chemical hydrolysis of captan occurs too rapidly for microbial degradation or accumulation of the parent compound to be significant.

2. The butoxyethylester of 2,4-dichlorophenoxyacetic acid is rapidly degraded to 2,4-D and butoxyethanol by all bacteria and fungi tested in the laboratory. Degradation of the resulting 2,4-D is a much slower process.

3. Under the conditions of our experiments methoxychlor is not degraded rapidly by bacteria.

4. The major metabolite of malathion degradation by the bacteria and fungi studied is the β-malathion monoacid.

5. In aqueous solution (pH 6.8-7.0, 27°C) containing low concentrations of malathion and low concentrations of malathion degrading bacteria, bacterial degradation can compete with chemical degradation.

6. The growth of the bacteria used to study carbaryl degradation is dependent on the rate of chemical hydrolysis of carbaryl to α-naphthol. In cultures containing α-naphthol the bacteria used in the carbaryl studies utilized α-naphthol as a sole carbon source.

7. The more water soluble pesticides -- atrazine, carbaryl, diazinon, malathion, and parathion -- were not sorbed by any of the bacteria or fungi tested; therefore, microbial sorption of these compounds would not be expected under natural conditions.

8. Sorption of methoxychlor and toxaphene by bacteria, fungi, and algae can be described by a partition coefficient and the process is rapidly reversible.

## SECTION IV

## RECOMMENDATIONS

1. The identification of the major microbial degradation products of malathion as the β-monoacid should facilitate an evaluation of the relative significance of chemical and microbial degradation in the environment.

2. Compounds naturally occurring in some waters may enhance microbial metabolism or degradation of pesticides. Information is needed on the effects of different concentrations and composition of these naturally occurring nutrients on microbial pesticide degradation.

3. Better procedures for determining the degradability of various pesticides by microorganisms in aquatic systems are needed to enable us to predict the fate of these compounds in aquatic systems.

4. Rates of microbial sorption of pesticides are needed. Methods for determining the sorption of pollutants by microorganisms should be developed in order to know more about the distribution of pollutants in an aquatic environment.

## SECTION V

## MATERIALS AND METHODS

### TEST MEDIA

A saturated solution of each pesticide was prepared by stirring the pesticide into basal salts solution and sterilized by passing the solution through a sterile 0.22-micron Millipore filter. The pesticides used in our studies with their sources, clean-up procedures, and water solubilities are listed in the Appendix. Replicate pesticide solutions of various concentrations were prepared by aseptically diluting the filtrate with sterile basal salts medium.

Payne and Feisal's basal salts medium (7) was used. All components of the medium were reagent grade chemicals purchased from J. T. Baker Company. The pH of the media used in all studies, except those with carbaryl and captan, was adjusted to pH 6.8 with 0.1 N HCl as determined by a Beckman Zeromatic meter. At pH 6.8 no chemical alteration of atrazine, malathion, diazinon, parathion, methoxychlor, 2,4-DBE, and toxaphene was detected during the course of experiments. Carbaryl and captan were not stable in the medium under alkaline or acid conditions.

### TEST ORGANISMS

The bacteria, fungi, and algae used in our studies, the areas from which they were isolated, and the pesticide studies for which they were used are given in Table 2. The following procedures were used for isolation and enrichment of the various classes of organisms studied.

#### Bacteria

Stock bacterial populations were obtained from water samples collected from four different aquatic sites and inoculated into nutrient broth (Difco) diluted 1:10 with water. These mixed cultures, separated according to their respective sites of origin, were initially inoculated into 1:10 nutrient broth containing a low concentration of the test pesticide.

After approximately one week the resultant populations were transferred to basal salts medium containing 12.6 mmol glucose and 0.1-1.5 µmol pesticide per liter and the

Table 2. MICROORGANISMS USED IN DEGRADATION AND SORPTION STUDIES

| Pesticide | Source | Organisms |
|---|---|---|
| Malathion | Potomac River | *Pseudomonas cepacia*<br>*Xanthomonas* sp.<br>*Commomonas terrigera*<br>*Flavobacterium meningosepticum* |
| Malathion | Shriner's Pond | *Aspergillus oryzae* |
| 2,4-DBE, Methoxychlor, and Toxaphene | Soya Creek | *Bacillus subtilis* |
| 2,4-DBE | Shriner's Pond | *Rhodotorula glutinis* |
| Methoxychlor and Toxaphene | Citrus Plant Effluent | *Flavobacterium harrisonii* |
| Carbaryl | Florida Pond | *Brevibacterium sulfureum*<br>*Pseudomonas ovalis*<br>*Bacillus megaterium*<br>*Flavobacterium lutescans* |
| Toxaphene and Methoxychlor | Starr's collection | Chlorella pyrenoidosa 395 |
| Toxaphene and Methoxychlor | Chicken Plant Effluent | *Aspergillus* sp. |

cultures were incubated on a gyratory shaker in an environmental chamber maintained at 28°C. When bacterial cultures indicated significant decreases in pesticide concentrations, an inoculum of the culture was transferred into fresh medium containing 5.05 mmol glucose and 1.2-3.0 µmol pesticide per liter.

Transfers into media containing lower concentrations of glucose and higher concentrations of pesticide were continued until a population of bacteria was obtained that grew in a medium containing the test pesticide as a sole carbon source. These enriched bacterial populations (Table 2) were lyophilized for degradation and sorption studies. Bacterial cultures not indicating a decrease in pesticide concentration after six weeks of transferring were discarded.

Fungi

Fungi from four field sites were isolated on Rose Bengal (Difco) plates and maintained on Saboraud's medium (Difco) slants. Fungal cultures were acclimated to pesticides in a manner similar to that used for bacteria by starting with high glucose concentration relative to test pesticides and proceeding to lower glucose concentrations relative to test pesticides. Fungal cultures were transferred approximately every two weeks and pesticide concentrations were monitored regularly for 12 weeks. Cultures not showing a decrease in pesticide concentration within 12 weeks were discarded.

Algae

Both axenic laboratory cultures of algae and algae collected from two field sites were used in sorption studies. The field samples also contained small numbers of bacteria and protozoa. The axenic laboratory cultures were grown in Bensen-Fuller medium containing 0.1% Hutner's trace elements (8) and incubated on a shaker at 15°C under 170 ft-c of continuous light. No enrichment procedures were employed.

## GAS LIQUID CHROMATOGRAPHY

All quantitative determinations were made using a Tracor MT-220 gas liquid chromatograph equipped with a nickel-63 high temperature electron capture detector. Pesticides were extracted from culture samples with Burdick and Jackson 2,2,4-trimethylpentane (distilled in glass) and no sample clean-up was needed. The nitrogen carrier gas flow was 120

ml/min; operating temperatures for the inlet port and detector were 170°C and 260°C. For carbaryl determination, the detector temperature was set at 225°C.

A short glass column (0.3m x 4mm ID) was used for rapid analysis of extracts. Columns were packed with 80-100 mesh Gas Chrom Q containing 3% silicone SE-30 (Applied Science Laboratories). Column temperatures for the various compounds are listed in Table 3.

Samples of 2,4-D were methylated with boron trifluoride/methanol (9) and extracted with isooctane prior to electron capture gas chromatographic analyses.

Malathion metabolites were methylated for gas liquid chromatography using a diazomethane procedure outlined in EPA report #EPA-R2-73-277 (10).

A linear response range was established for each pesticide and pesticide quantities were determined by peak height comparison (except for toxaphene) using standards with closely matched peak heights within the range of linearity. Toxaphene quantities were determined by peak area comparisons using a planimeter.

## THIN LAYER CHROMATOGRAPHY

Cultures containing malathion were adjusted to pH 2.0 with 1.0 N HCl and extracted with two 100-ml portions of chloroform. Products in the extract were separated by preparative thin layer chromatography using plates coated with silica gel. The developing solvent was hexane:acetic acid:ethyl ether (75:15:10) (11). Products were visualized by spraying a portion of the plate with the reagent of Menn et al. (12), 0.5% 2,6-dibromo-N-chloro-p-quinoneimine (DCQ) in acetone. $R_f$ values of the products were compared with the $R_f$ values of the standards.

Methoxychlor and products were extracted with hexane and plates were developed with ethyl ether/hexane (3:1). Elutions were visualized by spraying the plates with 0.5% diphenylamine and 0.5% zinc chloride in acetone, heating them at 110°C for 10 minutes, and exposing them to ultraviolet light for five minutes (13).

Carbaryl and products were extracted with methylene chloride and silica gel indicator plates were developed with benzene : 0.1 N ammonium hydroxide : ethanol (10:5:5).

Table 3. COLUMN TEMPERATURES (°C) FOR THE VARIOUS PESTICIDES AND METABOLITES INVESTIGATED

| Pesticides and Metabolites | Column Temperature (°C) |
|---|---|
| α-naphthol | 130 |
| 2,4-D (methylated) | 140 |
| Atrazine | 140 |
| Captan | 140 |
| β-Malathion Monoacid (methylated) | 150 |
| Carbaryl | 150 |
| Diazinon | 160 |
| Malathion | 170 |
| Parathion | 170 |
| 2,4-DBE | 190 |
| Methoxychlor-DDE | 190 |
| Methoxychlor-DDD | 190 |
| Toxaphene | 190 |
| Methoxychlor | 210 |

Visualization was accomplished by using a uv chromatographic viewer.

## MASS SPECTROMETRY

Pesticide degradation products (malathion monoacid, diethyl maleate, 0,0-dimethylphosphorodithioic acid, and methoxychlor-DDE) were identified using gas liquid chromatography-mass spectrometry. A Varian Aerograph Model 1532-B gas liquid chromatograph, a Finnigan 1055L quadrupole mass spectrometer having a jet separator, and a Systems Industry 150 digital computer were used. Sample spectra were compared with spectra of authentic samples of each compound.

## EXPERIMENTAL PROCEDURES

The following procedures were used for determination of degradation rates and products, and of distribution coefficients exhibited by the various organism populations. Bacteria were grown for 24 hours in nutrient broth diluted 1:10 with water spiked with the test pesticide prior to harvesting for study. The cultures were then centrifuged, washed three times with sterile dilution water, suspended in 100 ml of dilution water, and held at room temperature for an additional 24 hours to allow utilization of endogenous materials. These cultures were then used as inocula for media containing the pesticide as a sole carbon source for determination of degradation rates. The same procedure was used for yeasts except that Saboraud's medium diluted 1:10 was used rather than nutrient broth.

Inocula for fungi were suspensions prepared by agitating 10 ml of sterile water in a plate containing the sporulating fungi. A fungal medium was prepared containing basal salts and 0.278 mmoles glucose per liter. Replicate 500-ml flasks of the various weights of suspended fungi were prepared by measuring appropriate portions of an inoculated fungal medium, incubating on a gyratory shaker at 28°C for three days, and adjusting to a final volume of 210 ml with a basal salts solution containing a predetermined amount of pesticide.

Algal cultures were centrifuged, washed three times, and suspended in dilution water to use as inocula for sorption studies.

Numbers of viable cells of bacteria and yeasts were estimated by plate counts at zero hour and at each sampling time (14). Tryptone-glucose-extract agar (Difco) and Saboraud's agar (Difco) were used as the bacterial and yeast plating media respectively. Bacteria were incubated aerobically at 28°C for 48 hours and yeast, at 28°C for 72 hours. Fungi were separated from test cultures for dry weight determinations by filtering, first through tared pre-filters, then through tared 0.22 micron Nucleopore filters, and drying to a constant weight at 90°C. Dry weights of algae and bacteria used in sorption studies were determined by centrifuging and washing the organisms three times. The organisms were quantitatively transferred to tared beakers and dried to a constant weight at 90°C.

Pesticide determinations in degradation studies involved extraction of a portion of a test culture with isooctane and subsequent analysis by gas liquid chromatography. The size of the sample required depended on the concentration of pesticide, but usually 1 ml was sufficient. In sorption experiments, algal and bacterial samples were centrifuged and pesticide concentrations were determined in the supernatant. The filamentous fungi formed clumps and quickly settled to the bottom of flasks; they therefore posed no problem in sampling media without organisms.

## SECTION VI

## DEGRADATION STUDIES

Bacterial populations were found that could degrade five of the nine pesticides investigated: carbaryl, malathion, 2,4-DBE, methoxychlor, and parathion. Fungal populations were isolated that would degrade malathion and 2,4-DBE. The ability of bacteria or fungi to degrade captan was impossible to assess since the pesticide itself hydrolyzed so rapidly in solution.

Mixed populations were used since they often show a greater facility to acclimate than single species culture. A given species may not be able to initiate attack on a given compound but it may be able to use it for growth if another species initiates the attack.

The bacterial and fungal populations used in the degradation studies, although they are mixed, do not contain the total range of microorganisms present in a natural system. The initial isolation in the nutrient broth medium or Rose Bengal plates eliminated those species that could not grow in these media. The final isolation included primarily species that could grow in medium containing the pesticide as the sole carbon source.

The test cultures were monitored for decrease in pesticide concentration by extraction of whole cultures, i.e., both the organisms and medium, with isooctane and subsequent analyses by glc. Results were compared with those from controls containing pesticides but no microorganisms. If no change was noted in the pesticide concentration over a period of time, the pesticide was assumed to be non-degradable under the prevailing conditions.

Only a small sampling of natural populations were tested and a single set of carefully controlled conditions were used. With different populations or under different conditions rates may be different. However, testing a series of samples does give some insight into the general biodegradability of the compound. For example, in our system all test cultures rapidly degraded 2,4-DBE to butoxyethanol and 2,4-D. On the other hand, neither the bacterial nor the fungal populations tested degraded a detectable amount of atrazine, diazinon, or toxaphene. However, this does not say that under other conditions or with other microbial species degradation will not occur. It

only says that these compounds are not as readily degraded biologically as a compound such as 2,4-DBE.

## KINETICS

The rate of pesticide removal in the bacterial cultures may be described by the modified Monod expression (15) given in equation 1 in which [S] is the concentration of pesticide (μmol per liter); $\mu_m$ is the maximum specific growth rate ($hour^{-1}$); [B] is the concentration of bacteria (organisms per liter); Y is the yield factor or number of bacteria produced per μmol of pesticide; and $K_s$ is a constant numerically equal to the pesticide concentration at which $\mu = \frac{1}{2} \mu_m$.

$$-\frac{d[S]}{dt} = \frac{\mu_m [S][B]}{Y (K_s + [S])} \quad (1)$$

To determine $\mu_m$ and $K_s$ for the degradation of pesticides by bacteria a series of media with varying pesticide concentrations were inoculated with suspensions of washed organisms to give viable bacterial concentrations of $10^6$ - $10^8$ organisms per liter. Using a rearranged Monod equation (16)

$$\frac{[S]}{\mu} = \frac{[S]}{\mu_m} + \frac{K_s}{\mu_m} \quad (2)$$

experimental values [S]/μ were plotted as a function of [S]. The slope of the resulting plot, $1/\mu_m$, was determined by least squares analysis employing a computer program. $K_s$ was determined from the intercept ($K_s/\mu_m$).

Equation 1 takes into account the major factors influencing the rate of substrate utilization by batch cultures. At high substrate concentrations the equation reduces to

$$-\frac{d[S]}{dt} = \frac{\mu_m}{Y} [B] \quad (3)$$

Substrate removal follows pseudo first-order kinetics and is independent of substrate concentration.

At [S] much less than the value of $K_s$, equation 1 can be approximated by

$$\frac{d[S]}{dt} \simeq k[S][B] \quad (4)$$

where k is a second-order rate constant (liter organism$^{-1}$ hour$^{-1}$) for removal of pesticide by bacteria.

Equation 1, the more accurate description of substrate removal kinetics, requires knowledge of $\mu_m$, $K_s$, and Y, all of which may be determined from growth kinetics experiments. However, the very low solubility of many pesticides in water often precludes the range of experiments necessary to determine these parameters. The more simplified equation 4 would be useful if it were found to accurately define the kinetics for bacterial removal of pesticide. Malathion was used to establish the reliability of equation 4 because the insecticide was rapidly degraded by available bacterial populations and is soluble enough to permit work over a wide concentration range. We defined the term $[\mu_m/Y(K_s+[S])]$ from equation 1 as the second-order rate coefficient (k') and calculated k' using the kinetic data for $\mu_m$, $K_s$, and Y. The rate constant k in equation 4 was determined experimentally and compared with the calculated values of k'.

Values of k and k' (Table 4) are in agreement at low bacterial and malathion concentrations. Thus it was established that the second-order rate expression could be used for bacterial removal of a pesticide.

Equation 1 may also be used to describe the rate of pesticide removal by fungi. However, at the low concentrations of pesticides used in our studies very small increases in fungal biomass would be expected when the pesticide was the only external source of carbon. These small changes in fungal concentration could not be detected by our method of measurement (dry weight procedure) so the fungal biomass was assumed to be constant during the experiments. Values of $\mu_m$ and $K_s$ were not determined.

At low pesticide concentrations, though, equation 4 does describe the rate of removal of pesticide from solution by the fungi and can be used to determine the rate constant. The term [B] (concentration of fungi) is a constant for a given experiment.

## PESTICIDE DEGRADATION

Atrazine, diazinon, and toxaphene were not degraded by any of the bacteria or fungi tested.

Table 4. YIELD VALUES AND RATE CONSTANTS FOR REMOVAL OF MALATHION BY BACTERIA

| Malathion (μmol/ℓ) | Yield (org*/μmol) $\times 10^{10}$ | k (ℓ $org^{-1}$ $hr^{-1}$) $\times 10^{-12}$ | k′ (ℓ $org^{-1}$ $hr^{-1}$) $\times 10^{-12}$ |
|---|---|---|---|
| 0.0273 | 8.0 | 2.9 | 2.1 |
| 0.0273 | 8.0 | 2.5 | 2.1 |
| 0.21 | 1.8 | 1.2 | 8.6 |
| 0.21 | 2.3 | 2.2 | 6.8 |
| 0.273 | 3.0 | 3.5 | 5.1 |
| 0.273 | 3.0 | 1.9 | 5.1 |
| 0.33 | 4.1 | 3.4 | 3.6 |
| 0.33 | 2.6 | 3.3 | 5.7 |
| AVERAGE | 4.1 ± 2.3** | 2.6 ± 0.8** | 4.9 ± 2.1** |

*org = organism

**Standard Deviation

## Carbaryl

Carbaryl has been reported to be hydrolyzed both chemically (17,18) and biologically (19). Researchers, however, found it difficult to determine the relative extent of the two modes of degradation (19).

In our studies bacterial removal of carbaryl was found to be negligible, even when the bacterial population was increased from 1 x $10^9$ per liter to 1 x $10^{11}$ per liter (Figure 1). Growth of bacteria instead was dependent upon the rate of chemical hydrolysis of carbaryl to α-naphthol. The same bacterial population, grown in cultures containing α-naphthol, used the α-naphthol as a carbon source. Bacterial concentration increased 10 fold in 24 hours, removing all of the α-naphthol from the medium. Products of the bacterial degradation of the α-naphthol were 1,4-naphthoquinone and two unidentified compounds.

Hughes (18) reports that carbaryl is chemically hydrolyzed to α-naphthol in pond water under laboratory conditions. He also found a bacterium (Flavobacterium sp.), isolated from the pond water, to degrade the α-naphthol rapidly to o-hydroxycinnamic acid, salicylic acid, and an unidentified product. In Hughes' work, therefore, the bacteria cleaved the naphthalene ring.

Researchers working with soil fungi found that the fungi transformed carbaryl with no ring cleavage. Bollag and Liu (20) reported the degradation of carbaryl by a large number of soil fungi to naphthyl-N-hydroxy-methylcarbamate, 4-hydroxyl-1-naphthylmethylcarbamate, and 5-hydroxy-1-naphthylmethylcarbamate. The latter products may be the first step toward ring cleavage, since microorganisms usually hydroxylate a ring prior to cleavage.

## Malathion

Both bacteria and fungi were found to degrade malathion. The bacteria grew in the presence of malathion as the sole carbon source. As the bacterial concentration increased, the decrease in concentration of the insecticide was monitored by gas liquid chromatography of isooctane extracts of whole cultures, i.e, medium plus bacteria (Figure 2). All unmetabolized pesticide was therefore detected including any adsorbed onto the cell surface.

To study the kinetics of degradation, we first determined the maximum growth rate of bacteria on malathion.

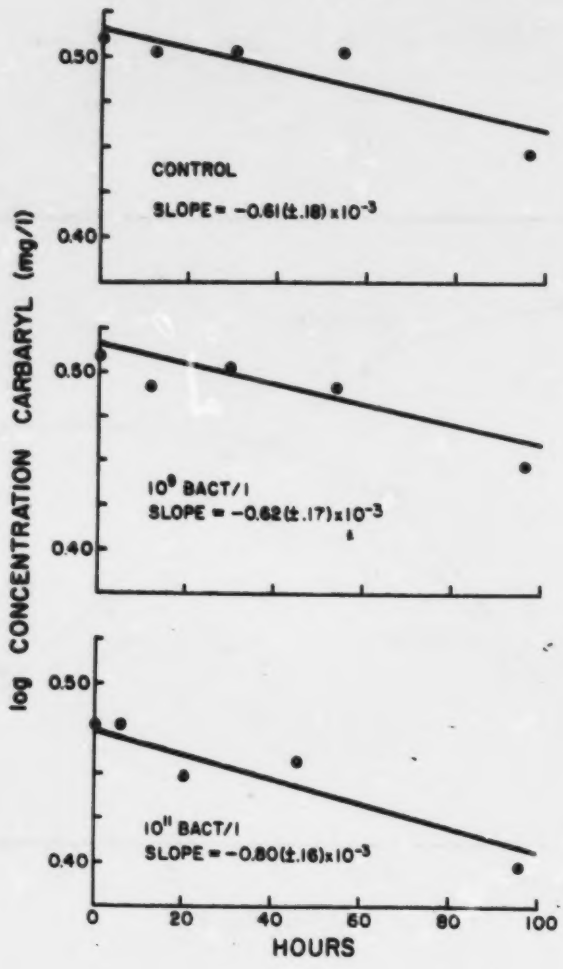



Figure 1. Decrease in carbaryl concentration with time.

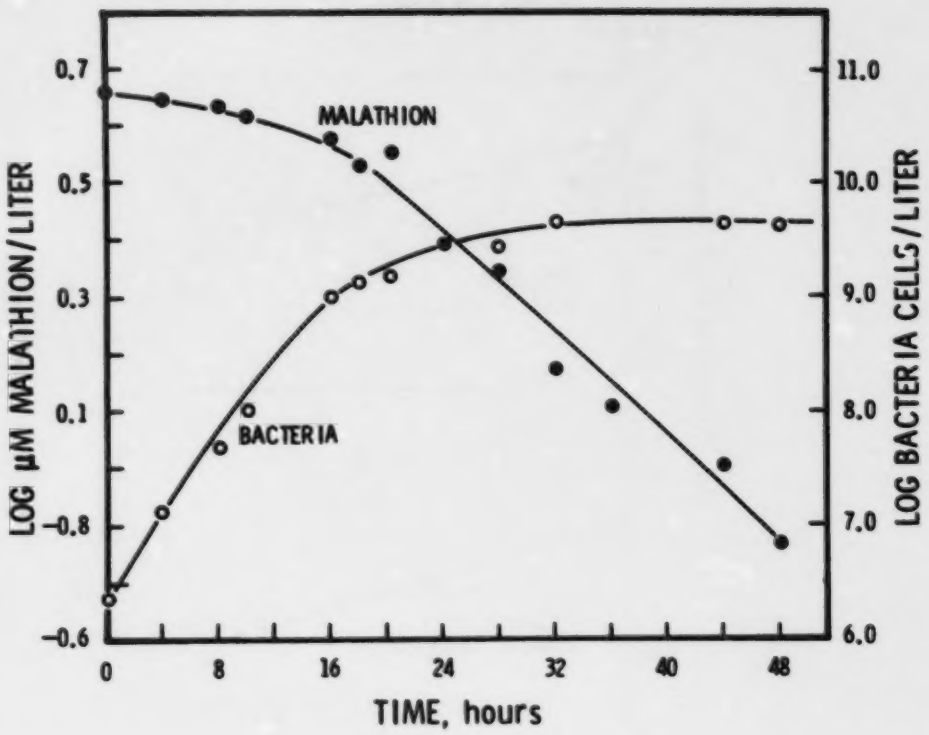



Figure 2. Growth of bacteria and decrease in malathion concentration.

Culture flasks containing malathion at concentrations ranging from 0.028 to 128 µmol/l were inoculated with suspensions of washed organisms sufficient to give viable bacterial concentrations of $10^6$ organisms per liter. The maximum rate of growth occurred in the medium having an initial malathion concentration of 13.6 µmol/l. Using equation 2, experimental values of $[S]/\mu$ were plotted as a function of [S] (Figure 3). $K_s$ was determined to be 2.17 µmol/l and $\mu_m$ to be 0.37 $hour^{-1}$.

At malathion concentrations one-fifth the value of $K_s$ or less, equation 4 describes the rate of bacterial removal of malathion. The constant k was found to range from 1.2 to $3.5 \times 10^{-12}$ liter $organism^{-1}$ $hour^{-1}$ (Table 4).

Whereas the bacterial population increased in concentration, no growth of the fungus, Aspergillus oryzae, was detected during the laboratory experiments. Fungal biomass was therfore assumed to be constant. The rate of removal of malathion from solution by A. oryzae may be described by equation 4, the second-order rate expression used in the bacterial rate studies. The second-order rate constant, k, for fungal degradation of malathion was $(1.10 \pm .66) \times 10^{-3}$ liter $mg^{-1}$ $hour^{-1}$. As indicated by values for k at very high organism concentration, based upon dry weight, malathion is removed by the bacteria approximately 5,000 times faster than by A. oryzae under similar conditions, i.e, malathion concentration, agitation rate, and temperature.

To further test these rate data, filter-sterilized river water containing malathion was inoculated with the fungi and bacteria found to degrade malathion. Under these conditions, the microbial half-life of malathion is given by equation 5. In this expression, $k_B$

$$t_{\frac{1}{2}} = \frac{0.693}{k_B[B] + k_F[F]}$$

and $k_F$ are the rate constants previously determined for bacteria and fungi respectively and [F] is the concentration of fungi (mg/l). The half-life measured under the experimental conditions was 2.2 hours, which is in good agreement with the calculated value of 2.5 hours.

The major metabolite of malathion degradation (97-99%) in both the bacterial and fungal systems was found to be the β-monoacid of malathion ( 5 ). The monoacid could be recovered quantitatively from both systems (Figures 4 and

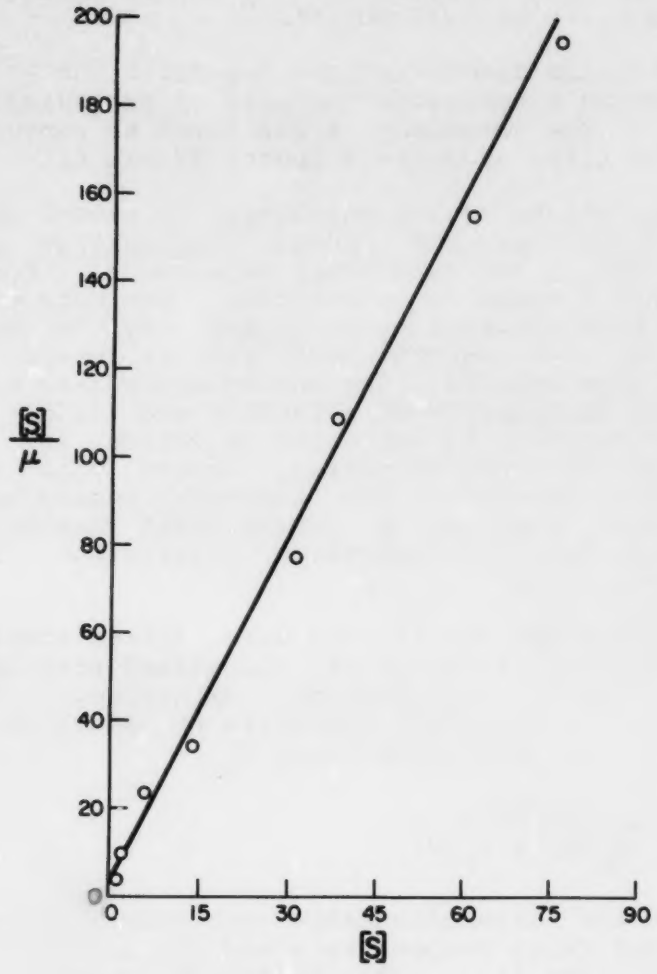



Figure 3. Lineweaver-Burke plot of specific growth rates and substrate concentrations for bacteria in malathion studies. [S] is concentration of malathion (μmol/l) and μ is specific growth rate ($hr^{-1}$).

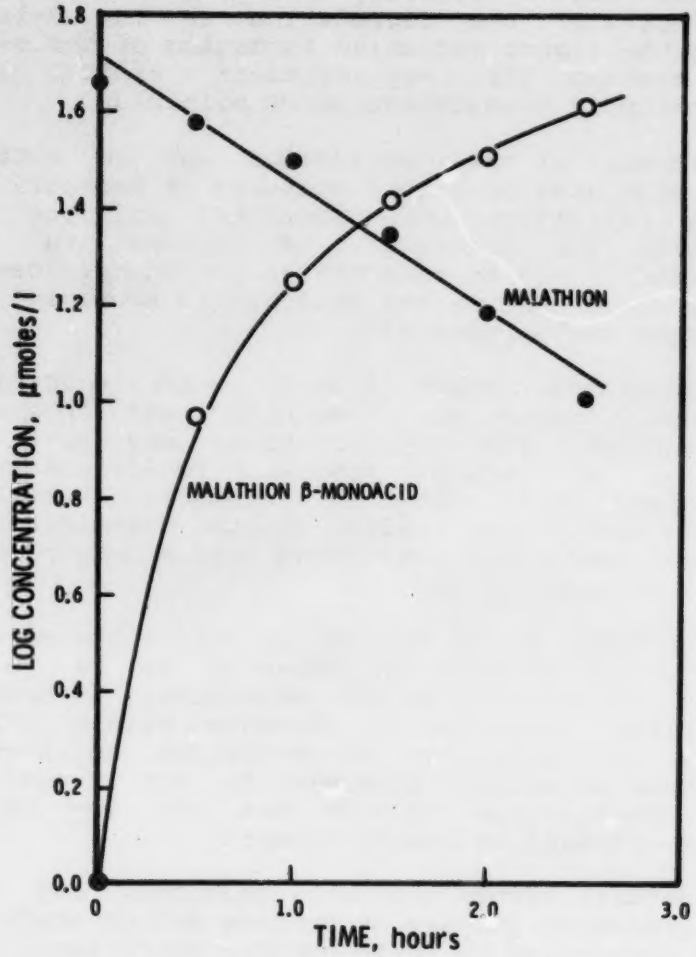



Figure 4. Formation of β-malathion monoacid in bacterial cultures.

5). Also detected were the malathion dicarboxylic acid in both fungal and bacterial systems and 0,0-dimethylphosphorodithioic acid and diethyl maleate in the bacterial system only. These products have been reported previously (21, 22) for bacterial and fungal degradation of malathion, but no attempt was made to identify the specific monoacid isomer. The specificity of the degradation to the β-isomer, as opposed to the almost exclusive formation of the α-isomer in chemical systems (5), may represent a typical pathway for the heterotrophic transformation of malathion.

To determine if the degradation was an extracellular reaction, filtrates of liquid cultures of bacteria and fungi containing malathion were incubated and the malathion concentration was monitored. No change in malathion concentration could be detected in six hours. Degradation, therefore, occurs within the cell and is probably catalyzed by the enzyme carboxyesterase.

The associated product of carboxyesterase activity would be ethanol. Since the β-monoacid was apparently not degraded further, the microorganisms may have used the ethanol as a carbon source. To determine if the microorganisms could grow on ethanol, bacteria were inoculated into basal salts medium containing 90 μmol/l ethanol, and fungi were introduced into a medium containing 2.1 mmol/l ethanol.

The ethanol concentration in both cultures was higher than the metabolite would be expected to be in cultures containing microorganisms and malathion. In both cultures the organisms increased in biomass within 48 hours. Apparently the malathion concentration and therefore the concentration of ethanol produced in the fungal cultures used for degradation studies was too low to produce a measurable increase in fungal biomass.

In a natural system physical, chemical, and biological removal processes (Figure 6) compete and interact and their rates are controlled by environmental conditions, which are characteristic of the individual aquatic system. For example, at pH 6.8-7.0 and 27°C, malathion does not readily hydrolyze; its half-life is about one month (5). However, at pH 9.0 and 27°C, the half-life of malathion is about 10 hours. The photolysis rate on the other hand, is dependent upon the concentration of humic acids. In water containing no humic acids, the photolysis half-life is 990 hours, whereas in water containing humic acids, the photolysis half-life is 15 hours (5). In the absence of humic acids, therefore, photolysis would not be expected to be the

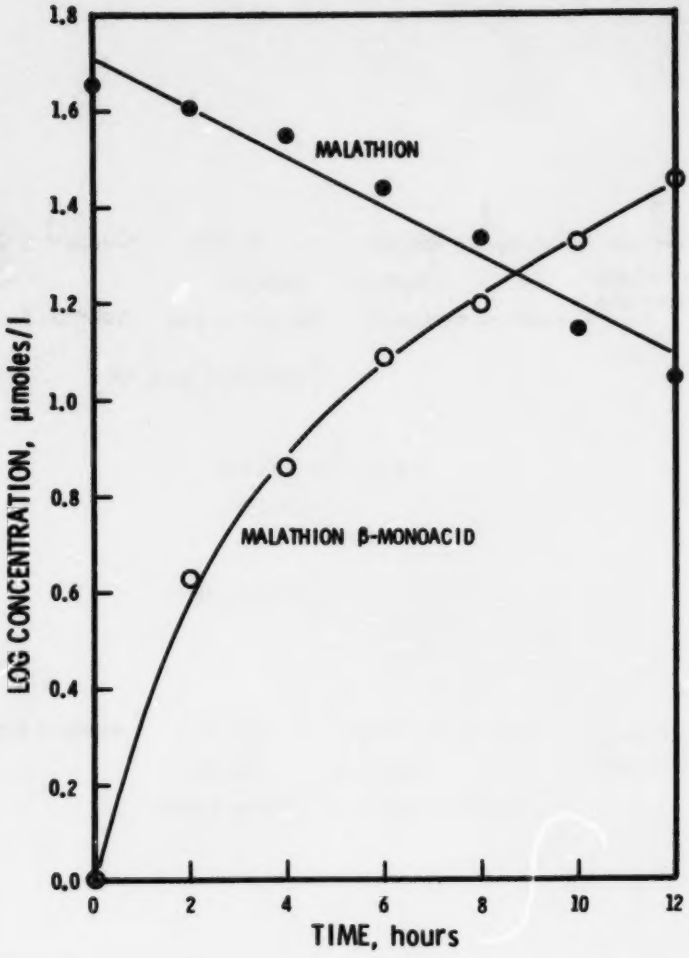



Figure 5. Formation of β-malathion monoacid in fungal cultures.

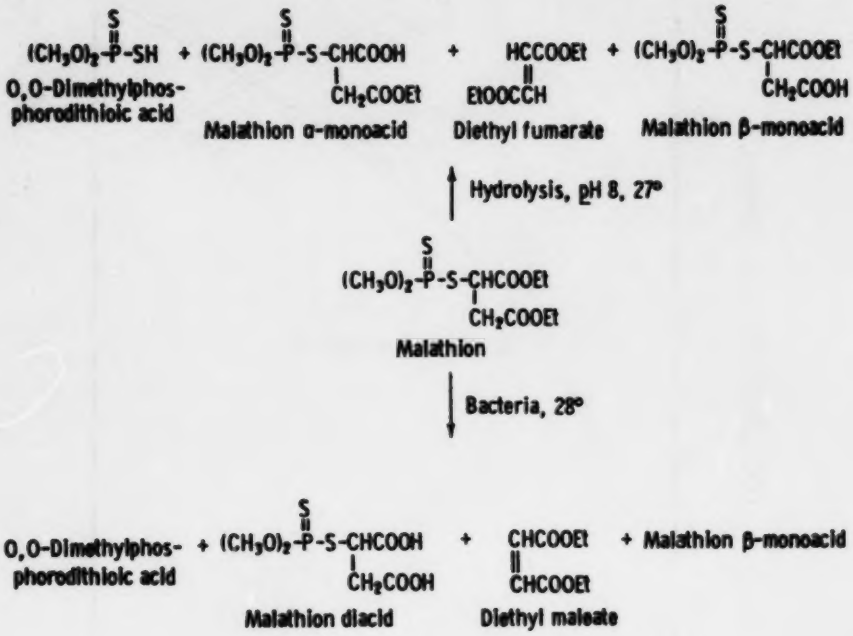



Figure 6. Comparison of chemical and microbial degradation products of malathion.

dominant degradation pathway. At low concentrations of malathion-degrading bacteria (2 x $10^6$/l) and low malathion concentration (3.3 µmol/l), the half-life of the pesticide was calculated to be 41 hours at 28°C. Therefore, in neutral waters (pH 6.8-7.0) containing little humic acids, bacterial removal may compete successfully. For the malathion degrading fungus to compete with the bacteria the fungal biomass would have to be 96 mg/liter (dry weight), a much higher concentration than one would expect in the environment.

Butoxyethyl Ester of 2,4-Dichlorophenoxyacetic Acid (2,4-DBE)

Bacillus subtilis grew in culture solution with the herbicide as a carbon source. Growth rates of B. subtilis at various concentrations of 2,4-DBE (0.156 to 15.6 µmol/l) were measured; $\mu_{max}$ was estimated to be 0.30 $hour^{-1}$ and $K_s$ to be 2.47 µmol per liter (Figure 7). At low concentrations of 2,4-DBE (0.1-1.0 µmol/l) and of B. subtilis (1 x $10^8$ org/l) the second-order rate expression (equation 4) describes the rate of removal of the herbicide from solution by bacteria. The constant k was (4.0 ± 1.3) x $10^{-11}$ liter $organism^{-1}$ $hour^{-1}$.

The major metabolite of ester degradation is 2,4-D. After 3 hours, 99% of the 2,4-DBE in a culture (initial concentrations 9.3 µmol/l 2,4-DBE, $10^{11}$ bacteria per liter) could be accounted for in the form of 2,4-D. Further degradation proceeded slowly. After 264 hours, 20% of the 2,4-D produced remained in the culture. Schwartz (23) and Aly and Faust (24) in their investigations also found that 2,4-D was persistent in an aqueous environment. Schwartz (23), following degradation of 2,4-D labelled at the 2-carbon of the acetic acid moiety, reported that no more than 37% of the acetic moiety disappeared within six months. Aly and Faust (24) found that 2,4-D persisted up to 120 days in lake waters aerobically incubated in the laboratory.

The metabolites of 2,4-D were not identified because the degradation pathway and products are assumed to be similar to those reported for soil microorganisms (25-35). In the studies with soil bacteria the phenoxyacetic acid ring was cleaved and metabolized to succinic acid.

Since the bacteria grew, but apparently did not use the 2,4-D as a carbon source, growth experiments were carried out to determine whether the organisms could use the other breakdown product, butoxyethanol. The bacteria could utilize the side chain for growth based on viable plate

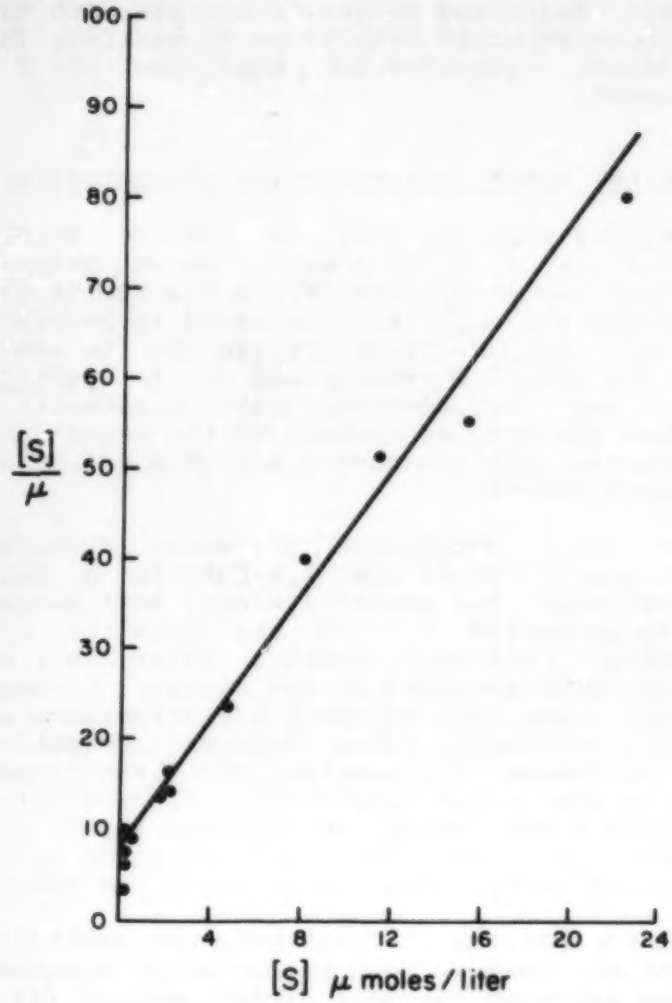



Figure 7. Lineweaver-Burke plot of specific growth rates and substrate concentrations for bacteria in butoxyethyl ester of 2,4-dichlorophenoxyacetic acid studies.

counts. In medium containing 210 µmol/l butoxyethanol as the carbon source the bacterial population increased 100-fold in 24 hours.

All four of the fungal populations tested including the yeast, Rhodotorula glutinis, degraded the ester, 2,4-DBE. For kinetic studies the yeast was selected because with it, population increases could be estimated by viable plate counts, a more convenient procedure than the dry weight method used for filamentous fungi. Results could therefore be compared more easily with the bacterial studies. R. glutinis, although it did degrade the pesticide, did not divide during the course of an experiment. When the medium was supplemented with 400 µmol/l butoxyethanol, sufficient carbon was available to permit the yeast to divide six times in 72 hours.

The second-order rate constant, k, was found to be (2.6 ± 2.0) x $10^{-9}$ liter $organism^{-1}$ $hour^{-1}$ in 2,4-DBE solution ranging from 0.9 µmol to 20.5 µmol per liter.

The mixed fungal populations (500 mg/l) in a solution containing 6.2 µmol/l 2,4-DBE converted 75-94% of the ester to 2,4-D within 15 minutes. During the same time period high concentrations ($10^{11}$ org/l) of the test bacterial populations converted 50-91% of the ester of 2,4-D. The short degradation times suggest the presence of a constitutive enzyme in all the test organisms.

When degradation rates for B. subtilis and R. glutinis were compared under equivalent conditions with the same organism concentration, the bacteria hydrolyzed the ester 100 times slower than did the yeast. However, the biomass, based on dry weight, of a single yeast cell (R. glutinis), was 100 times greater than that of a single bacterium (B. subtilis). The second-order rate constants, therefore, are nearly the same for the bacteria and yeast when compared on a biomass basis.

Further degradation of the 2,4-D was tested with all four fungal cultures. After a 48-hour incubation period in a solution containing 2,4-D as the sole carbon source, no growth could be detected and 88-99% of the initial acid remained. After 55 days, however, only 55-60% of the initial 2,4-D could be recovered. Degradation products were not identified.

Methoxychlor

Of the four bacterial populations screened for removal of methoxychlor, only one isolate, Flavobacterium harrisonii, caused a decrease in methoxychlor concentration in the culture within 216 hours. No significant growth of the bacteria was observed, as determined by viable plate counts, when methoxychlor was the sole carbon source; therefore, degradation rates were computed according to equation 2, assuming a constant bacterial population. The second-order rate constant, k, was determined to be (1.1 ± 0.56) x $10^{-13}$ $hr^{-1}$ in cultures containing methoxychlor at concentrations ranging from 0.006 to 0.15 µmol/l and bacterial concentrations of $10^{8}$ to $10^{9}$ per liter. This rate of degradation is slow compared to that observed with malathion and 2,4-DBE. Degradation did not occur in cultures until after 72 hours of acclimation, and ceased after 30% of the methoxychlor was degraded. Analyses of extracts of the cultures showed no further degradation after 192 hours. The rate constant, therefore, is descriptive only of the period of active degradation.

The main degradation product, identified by tlc, glc, and mass spectrometry was 2,2-bis(p-methoxyphenyl)-1,1-dichloroethylene, often referred to as methoxychlor-DDE. Our rate and product determination are in agreement with those reported by Mendel et al. (36). In their studies the bacterium, Aerobacter aerogenes, metabolized 65% of available methoxychlor to methoxychlor-DDE within 168 hours.

Since the Flavobacterium used in our study could not utilize methoxychlor as a source of carbon, enrichment of that organism in the field in the presence of methoxychlor is not expected. On the other hand, the bacteria in malathion and 2,4-DBE studies could utilize the pesticides as carbon sources, and would be expected to exhibit an enrichment in the presence of those pesticides.

Enrichment of microbial populations that degrade pesticides in the field has been suggested as an explanation for the decreasing persistence of certain pesticides observed upon successive application to field plots. This enrichment phenomenon can be a function of growth rate sustained in utilization of pesticides as nutrient sources. For example, Kearney (37) reports that chloropropham is more readily hydrolyzed than propham by a purified enzyme from Pseudomonas striata. However, the intact cells of P. striata degrade propham more readily and the resultant population is larger than when P. striata is cultured in the presence of chloropropham (38). Chloropropham, therefore,

is more persistent probably because of the slower growth rate of microorganisms on chloropropham.

## Captan

In preliminary experiments with captan and bacteria, the concentration of captan rapidly decreased in concentration in uninoculated controls (pH 5.6 to 8.0) until only a trace was detectable at 19 hours. Only a slight increase in the degradation rate was noted in the presence of bacteria. No further attempts were made, therefore, to study the microbial degradation of the fungicide.

## Parathion

One of our bacterial populations degraded parathion; however, because rate data for the bacterial degradation of parathion are in the literature, we did not determine degradation rates. Hsieh and Munnecke (39) assessed the capacity of microbial cultures to degrade parathion in water. In a chemostat, at a dilution rate of 0.05 $hr^{-1}$ and with a sufficient oxygen supply (580 mg per liter per hour) the bacteria removed parathion from solution at a continous rate of 500 ppm per hour. This is about 100 times higher than the rate of hydrolysis in 1 N sodium hydroxide.

## SECTION VII

## SORPTION OF PESTICIDES TO MICROORGANISMS

Some pesticides have been found to accumulate on or sorb to microorganisms. Several reasons for this phenomenon have been postulated. First, the water solubilities of most organic pesticides are quite low (See Appendix). Second, microorganisms have a very high surface area to mass ratio compared to aquatic organisms of higher trophic levels, e.g., for yeast the ratio is 9,100 $cm^2$ per gram and for Escherichia coli, a bacterium, 56,000 $cm^2$ per gram. Third, it has been suggested that since most pesticides are lipophylic, they are partitioned selectively onto surfaces containing surface lipids (40). The first and third reasons are only different ways of viewing the same phenomenon. All three reasons are dependent on the pesticide structure, which influences sorption of the molecule in microbial and soil systems.

We screened several microorganisms for their ability to sorb seven of the nine selected pesticides: a gram positive bacterium (Bacillus subtilis), a gram negative bacterium (Flavobacterium harrisonii), and three fungal populations. Captan was excluded because of its rapid chemical hydrolysis; 2,4-DBE was also excluded because all organisms tested converted 50-94% of the ester to 2,4-D within 15 minutes.

Although we did not screen algae along with the bacteria and fungi for their ability to sorb all the pesticides, we tested a green alga, Chlorella pyrenoidosa 395, along with the other microorganisms for extent of sorption of methoxychlor and toxaphene.

Sorption to the microorganisms was detected only in cultures containing the organochlorine pesticides -- methoxychlor and toxaphene. Equilibrium was reached within 16 hours. All the fungal populations screened sorbed these organochlorine compounds; Aspergillus sp. was chosen, however, for more extensive studies because its active spore formation made it convenient to transfer and to maintain in culture. Each culture of bacteria and algae was analyzed at intervals by centrifuging a sample of a culture, extracting it with isooctane, and determining the decrease in pesticide concentration in the supernatant. Fungal cultures were allowed to settle for one minute and samples of the supernatant were analyzed. Extraction of whole cultures accounted for all the pesticide; tlc and glc analyses

indicated no degradation of the organochlorine pesticides after 27 hours of incubation.

Uninoculated controls were also centrifuged, extracted, and analyzed in the same manner as were the samples. Any loss of pesticide in the controls, due to sorption on glassware or particles, was subtracted from the losses measured in the microbial cultures.

To determine whether the sorption of the pesticide was mediated by a metabolic process, the pesticides were also added to autoclaved cultures. These cells sorbed the pesticides at least as much as the viable cells. No metabolic process was therefore involved. Other researchers (41,42) report similar conclusions from their studies with bacteria and fungi and organochlorine pesticides.

## EQUILIBRATION TIME

The bacterial cultures reached equilibrium with both methoxychlor and toxaphene within 30 minutes, and no further change was detected over 24 hours. The algae equilibrated with the toxaphene within 10 minutes, but required 30 minutes with methoxychlor. The fungal system took the longest time to reach equilibrium -- two hours in the toxaphene medium and 16 hours in the methoxychlor medium. The fungi formed small clumps while growing. The same equilibration time was observed when the clump diameter was 5 mm as when it was 1 mm.

## EXTENT OF SORPTION

Sorption of pesticides to microorganisms may be represented by the empirically derived equation of Freundlich (43)

$$\frac{x}{m} = k\, c_e^{1/n} \qquad (6)$$

where x is the amount (mg) of pesticide sorbed to the microorganisms; m is the dry weight (mg) of the organisms; $c_e$ is the concentration of pesticide in the medium (mg/l) at equilibrium and k and 1/n are constants. The constant, 1/n, was determined from the slope of a log-log plot of x/m as a function of $c_e$. Since in all of our systems 1/n was about unity, the equation may be simplified to

$$k = \frac{x/m}{c_e} \qquad (7)$$

Arithmetic plots of x/m vs. $c_e$ showed a linear relationship (Figures 8 and 9). The graphs, along with the values of 1/n and k, (Table 5) were obtained using the least squares statistical computer program, MLAB (44), developed at the National Institutes of Health.

The term k, the slope of the arithmetic plot of x/m versus $c_e$, is a useful index for comparing the degree of sorption by the various classes or organisms (Table 5). The computed t values for the bacterial tests indicate a significant difference in the slopes at the 95% confidence level.

In systems in which 1/n is close to unity, k corresponds to the distribution coefficient, $K_d$, with a correction factor of $10^4$ to account for the different units used to obtain k. $K_d$ is merely a ratio of the amount of pesticide sorbed to the microorganisms (mg/mg) to the concentration of pesticide in water (in mg/mg), whereas k is calculated using units of mg/l for the concentration of pesticide in water.

The values of k for the four organisms and two pesticides are within an order of magnitude (Table 5). The greatest difference observed in ability to sorb the pesticides was between B. subtilis and F. harrisonii in the methoxychlor studies (B. subtilis, k = 0.048; F. harrisonii, k = 0.0012). If we assume B. subtilis to be a typical gram positive bacterium and F. harrisonii to be a typical gram negative bacterium, the difference in sorption cannot be explained by the lipid solubility of methoxychlor. The lipid content of the gram positive bacterial cell wall's dry weight is only 0-2%, as compared to 10-20% for the gram negative bacteria (45), yet the gram positive sorbed more methoxychlor. Shin et al. (46) studied adsorption of DDT by soil fractions. In their investigations, treating the soil with diethyl ether and ethanol for removal of lipoidal materials increased the adsorption of DDT to the soil, suggesting that other components of the soil play a larger role in the sorption of DDT than the lipoidal materials. If the cell wall of B. subtilis contains fewer polar groups than that of F. harrisonii, methoxychlor would be a more effective competitor with water for sites on the former.

All of the organisms, except B. subtilis, sorbed slightly more toxaphene than methoxychlor. However it is difficult to compare the degree of sorption for toxaphene itself since it is not a single compound. Toxaphene is a mixture of polychlorobicyclic terpenes and some components may be more tightly sorbed to the microorganisms than

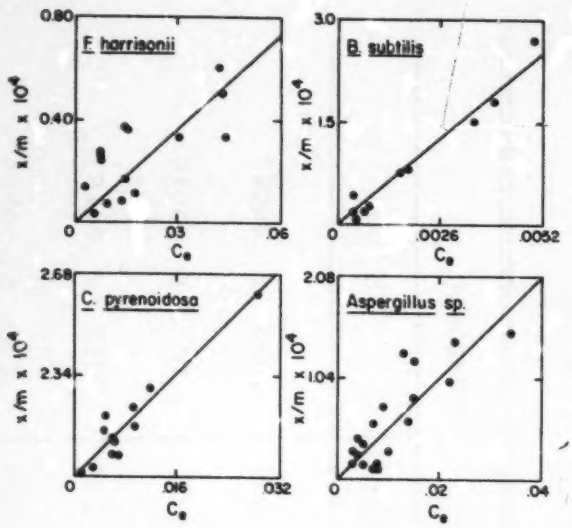



Figure 8. Sorption of methoxychlor by bacteria, fungi, and algae.

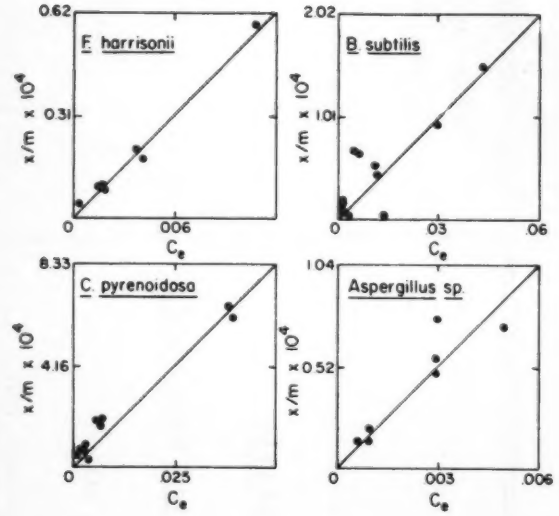



Figure 9. Sorption of toxaphene by bacteria, fungi, and algae.

Table 5. VALUES OF k AND 1/n FOR SORPTION OF METHOXYCHLOR AND TOXAPHENE TO VARIOUS MICROORGANISMS

| Organism | Methoxychlor | | Toxaphene | |
|---|---|---|---|---|
| | 1/n | k | 1/n | k |
| Bacillus subtilis | 1.2 | .048 ± .0022 | .71 | .0034 ± .00047 |
| Flavobacterium harrisonii | .81 | .0012 ± .00015 | 1.1 | .0052 ± .00016 |
| Aspergillus sp. | .91 | .0052 ± .00043 | .80 | .017 ± .0016 |
| Chlorella pyrenoidosa | .99 | .0084 ± .00052 | .79 | .017 ± .00088 |

others. Studies are underway to determine the degree of sorption of the various components of the pesticide.

Bailey and White (47) report an inverse relationship between the water solubility of a pesticide and the extent of adsorption to soils. However, the phenomenon was observed only within a family of compounds. We found that although toxaphene is ten times more water soluble than methoxychlor it is sorbed to a greater extent than methoxychlor by all organisms but B. subtilis. Our findings do not disagree with those of Bailey et al. (47) since these compounds are not of the same family. However, pesticides that are much more water soluble than toxaphene, e.g., atrazine, carbaryl, diazinon, malathion, and parathion, were not found to sorb to any detectable extent. This suggests that large differences in water solubility may affect microbial sorption.

## DESORPTION

When microorganisms that have sorbed pesticides move to aqueous environments containing little or no pesticide, they release some of the compound, redistributing it between the cell surface and the medium. We studied the desorption of methoxychlor and toxaphene by harvesting bacterial cells that had reached equilibrium in the pesticide solution and resuspending them in medium containing no pesticide. Samples were centrifuged, extracted, and analyzed as before for pesticide. The sorption was found to be a reversible process. Desorption equilibrium was achieved within the same short time as was equilibrium in the sorption studies; values for k were also the same. This ease of movement of the pesticide between the organisms and water would affect the distribution of the insecticides in the aquatic environment.

Veith and Lee (48) studied the desorption of toxaphene from Ottman Lake sediments. They suspended flocculent sediment (134 mg organic carbon per gram sediment) that contained pesticide in lake water. The pH of the supernatant was 8.3. After 10 days of leaching, the toxaphene content of the sediment was essentially unchanged. When pesticide free sediment was suspended in water containing toxaphene, sorption increased slowly over 200 days of incubation. The greatly different equilibration times for sediment and microorganism sorption of pesticides suggest different mechanisms of sorption.

## NATURAL WATERS

We determined k values for microorganisms in natural waters for comparison with our laboratory data. A water sample (pH 6.9) was collected from a river near High Shoals, Georgia, and centrifuged. Half of the supernatant was removed and replaced with distilled water containing methoxychlor (0.008 ppm final concentration). Direct microscopic examination of the sample showed algae (*Scenedesmus* sp. and *Chlorella* sp.), protozoa, and bacteria. No fungi were observed. The system equilibrated within 45 minutes. The overall k (average from all species) calculated from equation 7 was 0.0037, which is similar to that obtained previously for *F. harrisonii* and *Aspergillus* sp. but is one-tenth that of *B. subtilis* for the same pesticide.

Another water sample (pH 6.7) was collected from Chandler's pond near Athens, Georgia. It was centrifuged and half of the supernatant was replaced with distilled water containing toxaphene (final concentration, 0.047 ppm). The sample contained about twice as many algae as the High Shoals sample as determined by microsccpic and direct observation. Microorganisms present were green algae (*Arthospira* sp., *Phytoconis* sp., and a few *Chlorella* sp.), bacteria, ciliates, and diatoms. The system equilibrated within one hour and k was determined to be 0.0067. This is similar to the value obtained for *F. harrisonii* and *B. subtilis* and one-half that observed in the laboratory for *Aspergillus* sp. and *C. pyrenoidosa*.

The algae formed the largest segment of the microorganism population in both field samples; total algal biomass for the two samples were 100 mg/l (High Shoals) and 200 mg/l (Chandler's Pond). The calculated k values for *Chlorella* in the laboratory media containing methoxychlor and toxaphene are in good agreement with those obtained for the total microorganism populations in the field samples. Although only two field sites were tested, laboratory data with isolates appear to give a reasonable approximation of sorptive behavior in a mixed natural population.

Appendix. SOLUBILITIES OF THE SELECTED PESTICIDES IN BASAL SALTS MEDIUM

| Pesticide | Solubility in Basal Salts Medium (ppm) | Clean-up Procedure | Source |
|---|---|---|---|
| Atrazine | 30.0 | -- | Ciba-Geigy |
| Captan | 5.0 | -- | Matheson, Coleman and Bell |
| Carbaryl | 43.0 | Recryst. with ethyl ether | Union Carbide |
| 2,4-DBE | 8.4 | Redistilled | Amchem Company |
| Diazinon | 36.0 | -- | Ciba-Geigy |
| Malathion | 100.0 | -- | American Cyanamid |
| Methoxychlor | 0.05 | Recryst. with ETOH | Ciba-Geigy |
| Parathion | 19.0 | Redistilled | Monsanto |
| Toxaphene | 0.2 | -- | Hercules |

*All pesticides except captan were gifts of the companies.

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